THE EFFECTS OF ANTUITRIN S AND SHEEP PITUITARY EXTRACT ON THE FEMALE LIZARD, ANOLIS CAROLINENSIS

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Introduction

The relationship between the pituitary and the female genital system of the reptile is only partially understood. Ovulation in the snake, *Xenodon merremi*, resulted after five homoplastic pituitary implants (Houssay, 1931). Ovulation was induced in *Anolis carolinensis* after five homoplastic pituitaries were implanted and in one case after three frog pituitaries were implanted (Evans, 1935). Forbes (1934) reports hypertrophy of the genital system of young alligators with whole gland sheep pituitary extract.

Since homoplastic pituitary implants induce ovulation in reptiles, the present study was made in order to learn whether mammalian pituitary extracts would also induce ovulation.

The oviduct of the normal female of *Anolis carolinensis* is divided into five general regions: the infundibulum, the tube, the albumen-secreting portion, the uterus, and the vagina (Giacomini, 1893). The epithelial cells of the albumen-secreting portion are cuboidal and seem to be the only glands present, there being none beneath the mucous layer. Special glands, however, exist in the submucous region of the uterus. These connect with the lumen of the uterus by means of ducts.

Materials and Description

Antuitrin S Series

Fifty-five females of *Anolis carolinensis* received injections of Antuitrin S (Parke Davis, Series 3024898). The first injections were administered on November 22, 1933, the last on April 1, 1934. Some females received more injections than others and at varying intervals, but the dosage in all cases was .02 cc. diluted with two or three parts of cold-blooded Ringer's solution. This dose represents the maximum that was safe to use. Larger dosage often proved fatal. Twenty-five females served as controls and were kept under the same conditions as the injected animals.

Internal changes brought about by the injections were largely con-

fined to the ovaries and oviducts. Figure 1*a* shows the control (on the left) as compared to 1*b* from a female which received three daily injections, was then rested for 30 days, followed by six more injections, then another rest of 7 days followed by two injections. She was killed 24 hours later, February 28, 1934, together with the control shown in Fig. 1. Figure 1*c* shows the condition produced in a female which received twelve daily injections and then was killed, 24 hours later, March 15, 1934. These two particular cases are representative of the results obtained in the 55 females, so it seems unnecessary to illustrate other cases.

Reference to Fig. 1 reveals the alteration produced in the oviducts by injections. In the control the oviduct appears as a continuous straight tube which becomes slightly larger in the region of the uterus. Figures 1*b* and 1*c*, however, show the oviduct very much enlarged and thrown into many folds. The albumen-secreting portion becomes greatly lengthened but its walls remain relatively thin. The uterus, on the contrary, increases more in circumference than in length. This increase in diameter is brought about partly by a thickening of the walls due to the greatly hypertrophied glands lying beneath the mucous lining of the lumen. The infundibulum (*i*), albumen-secreting portion (*al*), and the uterus (*u*) are well shown.

The lumen of the infundibular region is usually closed by the temporary fusion of the mucous walls but, under the influence of the extract, the lumen becomes patent. The epithelial cells lining the tube region are not visibly hypertrophied by the hormone. The mucosa of the albumen-secreting portion shows considerable response. The individual cells change in shape from cuboidal to columnar.

EXPLANATION OF PLATE I

Abbreviations: *al*, albumen-secreting portion of oviduct; *g*, glandular area of uterus or shell-secreting portion of oviduct; *i*, infundibulum of oviduct; *l*, lumen; *m*, smooth muscle layer of oviduct; *s*, mucous layer of oviduct.

All figures are of *Anolis carolinensis*.

FIG. 1. 4¾ ×. Ovary and oviduct. (*a*) Control. Killed February 28, 1934. (*b*) Received injections of Antuitrin S with intervals of rest as follows: three daily injections, 30 days rest, 6 daily injections, 7 days rest, 2 daily injections. Killed February 28, 1934. (*c*) Received 12 daily injections of Antuitrin S. Killed 24 hours later, March 15, 1934. (*d*) Received 12 injections of whole gland sheep pituitary extract at 36-hour intervals. Killed 24 hours later, March 15, 1934.

FIG. 2. 210 ×. Control. Cross-section of albumen-secreting portion of the oviduct.

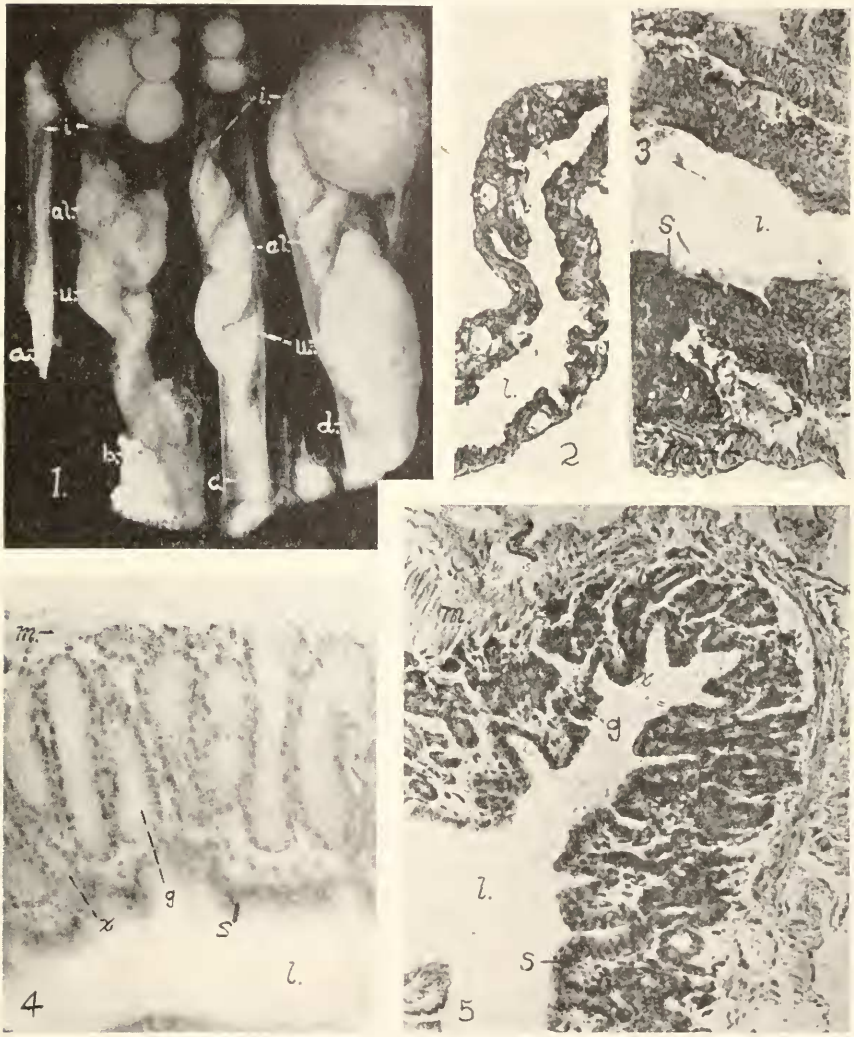
FIG. 3. 210 ×. Same as *d* in Fig. 1. Cross-section of albumen-secreting portion of the oviduct.

FIG. 4. 210 ×. Same as *d* in Fig. 1. Cross-section of uterus. Note duct from glandular area at *x*.

FIG. 5. 210 ×. Control. Cross-section of uterus. Note duct from glandular area at *x*.

The uterus shows a curious contrast to the albumen-secreting portion in its response to the injections. The superficial cells of the mucosa are not altered in shape but remain cuboidal. The so-called

PLATE 1



shell-secreting glands which lie between the mucous layer and the muscle layer become much enlarged.

The ovary is definitely affected by Antuitrin S as Figs. 1*a*, 1*b*, and 1*c* bring out clearly. Figure 1*c* shows approximately 100 per cent hypertrophy as compared with the control. In Fig. 1*b*, however, the ovary is at least four times larger than the control.

It is interesting to note that eleven injections in groups of 3, 6, and 2, with several days interval between injections, produced a greater hypertrophy of both ovary and oviduct (1*b*) than was secured when one injection was given daily for twelve days (1*c*). Seasonal effects can be ruled out since 1*b* was killed two weeks earlier than 1*c*.

No matter what combination of injections and rest intervals was used, Antuitrin S failed to induce egg-laying.

Sheep Pituitary Series

Twenty-five females of *Anolis carolinensis* were injected with sheep pituitary (whole-gland pituitary extract [1]) at varying intervals between December 1, 1933 and April 1, 1934 and with a varying number of injections. A single dose was always .02 cc. diluted as for Antuitrin S. The same controls were used.

The use of sheep pituitary gave results which were more satisfactory in that complete ovulation was induced. On March 23, 1934 two eggs were laid and on March 24, two more. A fifth appeared on April 11. In these particular cases, twelve injections of sheep pituitary were given at an average of 36-hour intervals, the last injection being on March 2, 1934. All of the 25 females that received the sheep pituitary responded by a great hypertrophy of the ovaries and oviducts. Many cases showed, however, that the eggs which were ready to be laid were retained in the ovary. Such females had unusually large abdomens, moved about very little, and seemed to be in a lethargic state. In these cases the eggs were very slowly resorbed so that by the first of August the ovaries were reduced to the size of that shown in Fig. 1*c*. The bright orange color of the partially resorbed eggs made them easily distinguishable from young ova of the same size which were always white.

Figure 1*d* shows a typical case of induction. This particular animal was killed March 15, 1934, 24 hours after receiving twelve injections at 36-hour intervals. The large egg is almost ready to leave the ovary. The infundibulum has surrounded the egg, enveloping it like a transparent membrane. The latter is visible in the fresh condition only because of its blood vessels, which form a network over the egg (not visible in the photograph). The same figure shows clearly the infundibulum, albumen-secreting portion, and the uterus. Comparison with the normal oviduct at the time of ovulation (not shown) makes it seem certain that this figure represents a condition homologous to that just prior to normal ovulation.

Figure 1*d* represents, then, the maximum degree of hypertrophy which was obtained in our injected series. The uterine portion is

[1] Kindly supplied by Dr. Oliver Kamm of Parke Davis Company.

especially large. This hypertrophy is due primarily to the increase in the size of the glands which lie in the submucous region. These are larger in all dimensions than the same glands in Fig. 1*b* or 1*c*. Figure 4 shows a section of this uterus in greater detail. The glandular cells are palisade in shape and are filled with a highly refractive granular substance. The nuclei lie at the outer periphery of the cells and away from the lumen of the gland. The cells have become so enlarged that the free secreting surfaces of opposite cells abut one another, thus closing the lumina of the glands.

Non-sexual Effects of Both Antuitrin S and Sheep Whole Gland Extract

These effects made their appearance after the third or fourth injection and continued for at least four months after the last injection. Both extracts caused the females to differ from the controls in that (1) their appetites were greater and while they ate more they remained much thinner; (2) general activity and speed of movement were greater; (3) moulting occurred oftener.

Summary

Between November 1933 and April 1934, 55 females of *Anolis carolinensis* were injected with Antuitrin S, while 25 were injected with whole gland sheep pituitary extract. Twenty-five females served as controls. The dosage was .02 cc. of either extract diluted with cold-blooded Ringer's solution.

Both extracts caused hypertrophy of the ovaries and oviducts but ovulation and egg-laying were induced only with sheep pituitary. Moreover, in many females which received sheep extract the ovaries contained mature ova that were not laid but were slowly resorbed during the ensuing spring and summer.

With both extracts the epithelial cells lining the albumen-secreting portion of the oviduct changed from cuboidal to columnar, while those of the epithelium of the uterus were very little affected by the injections. The deep-lying shell glands of the uterus, however, were greatly enlarged.

Injected animals ate more, were more active, and moulted oftener than controls.

I wish to thank Professor Leigh Hoadley and Professor Alden B. Dawson for their kind help and valuable criticism during the course of this investigation.

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